Store at -20°C

#8339

HER/ErbB Family Antibody Sampler Kit

1 Kit
(8 x 20 µL)

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
EGF Receptor (D38B1) XP® Rabbit mAb	4267	20 µl	175 kDa	Rabbit IgG
HER2/ErbB2 (D8F12) XP® Rabbit mAb	4290	20 µl	185 kDa	Rabbit IgG
HER3/ErbB3 (D22C5) XP® Rabbit mAb	12708	20 µl	185 kDa	Rabbit IgG
HER4/ErbB4 (111B2) Rabbit mAb	4795	20 µl	180 kDa	Rabbit IgG
Phospho-EGF Receptor (Tyr1068) (D7A5) XP® Rabbit mAb	3777	20 µl	175 kDa	Rabbit IgG
Phospho-HER2/ErbB2 (Tyr1221/1222) (6B12) Rabbit mAb	2243	20 µl	185 kDa	Rabbit IgG
Phospho-HER3/ErbB3 (Tyr1289) (D1B5) Rabbit mAb	2842	20 µl	185 kDa	Rabbit IgG
Phospho-HER4/ErbB4 (Tyr1284)/EGFR (Tyr1173) (21A9) Rabbit mAb	4757	20 µl	180 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions, and additional application protocols.

Description: The HER/ErbB Family Antibody Sampler Kit provides an economical means to evaluate the HER/ErbB Family, including the phosphorylation of EGFR, HER2/ErbB2, HER3/ErbB3, and HER4/ErbB4. The control antibodies to each family member are also included. The kit contains enough antibody to perform two western blot experiments with each primary antibody.

Background: The epidermal growth factor (EGF) receptor is a transmembrane tyrosine kinase that belongs to the HER/ErbB protein family. Ligand binding results in receptor dimerization, autophosphorylation, activation of downstream signaling, internalization, and lysosomal degradation (1,2). The GRB2 adaptor protein binds activated EGFR at phospho-Tyr1068 (3).

The ErbB2 (HER2) proto-oncogene encodes a 185 kDa transmembrane, receptor-like glycoprotein with intrinsic tyrosine kinase activity (4). While ErbB2 lacks an identified ligand, ErbB2 kinase activity can be activated in the absence of a ligand when overexpressed and through heteromeric associations with other ErbB family members (5). Amplification of the ErbB2 gene and overexpression of its product are detected in almost 40% of human breast cancers (6). The major autophosphorylation sites in ErbB2 are Tyr1248 and Tyr1221/1222; phosphorylation of these sites couples ErbB2 to the Ras-Raf-MAP kinase signal transduction pathway (4,7).

HER3/ErbB3 is a member of the ErbB receptor protein tyrosine kinase family, but lacks tyrosine kinase activity. Tyrosine phosphorylation of ErbB3 depends on its association with other ErbB tyrosine kinases. Upon ligand binding, heterodimers form between ErbB3 and other ErbB proteins, and ErbB3 is phosphorylated on tyrosine residues by the activated ErbB kinase (8,9). There are at least 9 potential tyrosine phosphorylation sites in the carboxy-terminal tail of ErbB3. These sites serve as consensus binding sites for signal transducing proteins, including Src family members, GRB2, and the p85 subunit of PI3 kinase, which mediate ErbB-downstream signaling (10). Both Tyr1222 and Tyr1289 of ErbB3 reside within a YXXM motif and participate in signaling to PI3 kinase (11).

HER4/ErbB4, like other family members, has four ectodomains, a single transmembrane domain, and a cytoplasmic tail containing the active tyrosine kinase domain (12). By binding to neuregulins and/or EGF family ligands, ErbB4 forms either a homodimer or heterodimer with other ErbB family members, which results in receptor activation and signaling (12). ErbB4 is ubiquitously expressed with the highest expression occurring in the brain and heart. The expression of ErbB4 in breast cancer, pediatric brain cancer, and other types of carcinomas has been reported, suggesting that ErbB4 expression is involved in both normal tissue development and carcinogenesis (12).

Specificity/Sensitivity: Each antibody in the HER/ErbB Family Antibody Sampler Kit recognizes only its specific target. The antibodies do not cross react with other HER/ErbB family members.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with a fusion protein containing the cytoplasmic domain of human EGF receptor, a synthetic peptide corresponding to residues near the amino terminus of human ErbB2 protein, the carboxy terminus of human ErbB3 protein, or the carboxy-terminus of human ErbB4 protein. Activation state monoclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Tyr1068 of human EGF receptor protein, tyrosines 1221/1222 of human ErbB2 protein, Tyr1289 of human HER3/ErbB3 protein, or Tyr1284 of human ErbB4 protein.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibodies.*

Recommended Antibody Dilutions:
Western blotting 1:1000

Please visit www.cellsignal.com for validation data and a complete listing of recommended companion products.

Background References:

(1) Hackel, P.O. et al. (1999) *Curr Opin Cell Biol* 11, 184-9.
(2) Zwick, E. et al. (1999) *Trends Pharmacol Sci* 20, 408-12.
(3) Rojas, M. et al. (1996) *J Biol Chem* 271, 27456-61.
(4) Muthuswamy, S.K. et al. (1999) *Mol Cell Biol* 19, 6845-57.
(5) Qian, X. et al. (1994) *Proc Natl Acad Sci U S A* 91, 1500-4.
(6) Dittadi, R. and Gion, M. (2000) *J Natl Cancer Inst* 92, 1443-4.
(7) Kwon, Y.K. et al. (1997) *J Neurosci* 17, 8293-9.
(8) Yarden, Y. and Sliwkowski, M.X. (2001) *Nat Rev Mol Cell Biol* 2, 127-37.
(9) Guy, P.M. et al. (1994) *Proc Natl Acad Sci U S A* 91, 8132-6.
(10) Songyang, Z. et al. (1993) *Cell* 72, 767-78.
(11) Kim, H.H. et al. (1994) *J Biol Chem* 269, 24747-55.
(12) Carpenter, G. (2003) *Exp Cell Res* 284, 66-77.

U.S. Patent No. 5,675,063

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Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

#8339

Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, mix.
3. **1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH_2O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, mix.
5. **10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH_2O, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH_2O, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl for a 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time. **NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.

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